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ORIGINAL ARTICLE

# New spectrophotometric methods for the determination of sulfadoxine by the formation of Co(II) complexes

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Spectrophotometry;
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Co(II) complex

**Abstract** New sensitive spectrophotometric methods are developed for the determination of sulfadoxine (SFD). Proposed methods are based on the reaction of drug with aryl aldehyde followed by Co(II) chloride in the acidic medium. The reaction of aryl aldehyde with drug results in the formation of Schiff base which acts as a ligand for the formation of complex with Co(II). Different aryl aldehydes are tested, out of which *p*-dimethylaminobenzaldehyde (used in method A) and vanillin (used in method B) gave stable green colored complexes. The complexes formed due to *p*-dimethylaminobenzaldehyde and vanillin are measured at 672 and 665 nm for method A and method B, respectively. The molar absorptivity and Sandell's sensitivity of method A and method B are $0.1264 \times 10^4$, 0.2453 and $0.6982 \times 10^4$, 0.4444, respectively. The optical reaction condition and their analytical parameters are evaluated. Both the methods are free from interference of the ingredients such as lactose, sucrose and starch, thus it is successfully applied to pharmaceutical formulations.


## 1. Introduction

Sulfadoxine is chemically 4-amino-*N*-(5,6-dimethoxypyrimidin-4-yl)benzene-1-sulfonamide belonging to the class of drug known as sulfanilamides. It is mainly used for the treatment or prevention of malaria and also used as anti-infective agent (British Pharmacopoeia, 2008). The enhanced clinical use of Sulfadoxine necessitated the development of new methods for the determinations of Sulfadoxine. Literature survey revealed the estimation of Sulfadoxine in pharmaceutical formulations by various techniques such as spectrophotometry (Sharma and Sharma, 2011; Sangita et al., 2010; Onah and Odeiani, 2002; Green et al., 1965; Parimoo, 1987; Raghuveer et al., 1993; Ansari et al., 2008), liquid chromatography (Lindkvist et al., 2009; Guo-Zhen et al., 2006) electrophoresis (Soto-Chinchilla et al., 2007), potentiometry (Kharitonov and Gorelov, 2000), and RP-HPLC (Arayne et al., 2010). Among the reported techniques, visible spectrophotometry is a technique of choice employed in quality control laboratories of many developing countries because of its inherent simplicity, sensitivity and cost-effectiveness. Therefore, developing selective and sensitive methods using visible spectrophotometry is of paramount importance.

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Quite a few visible spectrophotometric methods have been developed for the quantification of Sulfadoxine in pharmaceuticals. However, many of these methods suffer from one or more disadvantages such as critical optimum conditions, heating, and extraction using organic solvent, narrow linear dynamic range, low sensitivity and poor selectivity. To overcome these problems, new and simple spectrophotometric methods were planned to be developed for the determination of Sulfadoxine.

Several spectrophotometric methods have been reported for the determination of various drugs by condensing it with aldehydes such as *p*-dimethylaminobenzaldehyde, *p*-dimethylaminocinnamaldehyde, and vanillin (Srihari et al., 2011, Alhemiary and Saleh, 2012; Oga, 2010; Revanasiddappa et al., 2012; Kapse et al., 2006). But, the Schiff bases formed in such cases showed absorption peak at lower wavelength. However, several Schiff base ligands have been proposed as spectrophotometric reagents for metal ions (Afkhami et al., 2011; Narayana et al., 2009).

In view of the enhanced applications of sulfadoxine, the study of new methods for its determination became very important and useful for quality control laboratories. So in the present investigation, we have developed new spectrophotometric methods for the determination of sulfadoxine involving the formation of Schiff bases with *p*-dimethylaminobenzaldehyde and vanillin, then formation of colored Co(II) complexes.

## 2. Materials and methods

### 2.1. Apparatus

A UV–Visible spectrophotometer (SHIMADZU, UV 2550, Japan) with 1 cm quartz cells was used for the absorbance measurements.

### 2.2. Reagents and solutions

All solutions were prepared with double distilled water. Chemicals used were of analytical reagent grade. Solutions of

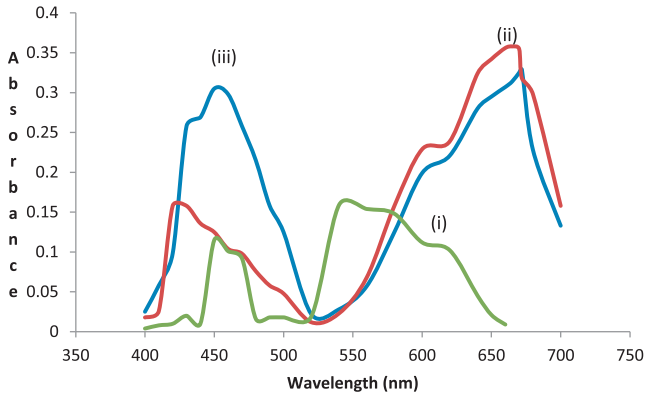
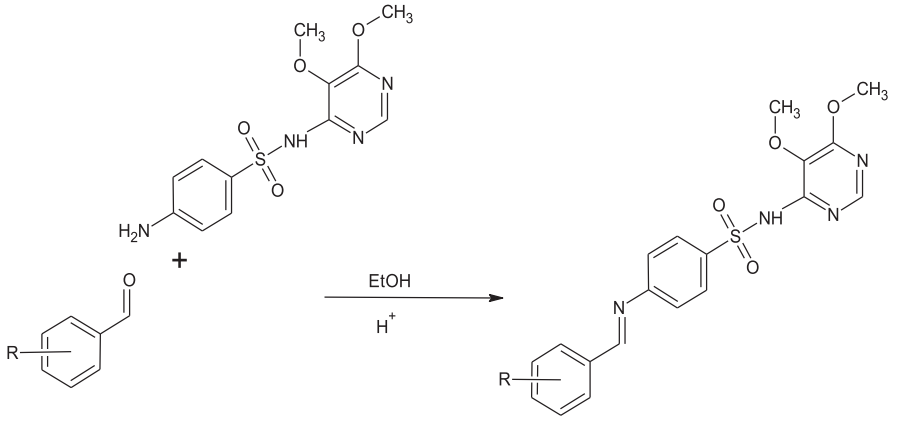



**Figure 1** Absorption maximum for (i) Co(II), (ii) method A, and (iii) method B.

*p*-dimethylaminobenzaldehyde and vanillin ($5 \times 10^{-4}$ M) were prepared by dissolving 500 mg in 100 mL ethanol and $CoCl_2$ $6H_2O$ ($1.54036 \times 10^{-4}$ M) was prepared by dissolving 0.02 g in 100 mL of water. About 100 mg of pure sulfadoxine weighed accurately and dissolved in 5 mL of (2 M) Hydrochloric acid and diluted to 100 mL with ethanol (1000 μg $mL^{-1}$). The stock solution was diluted approximately to get working concentration.

### 2.3. Determination of sulfadoxine by using *p*-dimethylaminobenzaldehyde (method A)

Aliquots of Sulfadoxine containing 40.00–100 μg $mL^{-1}$ were transferred into a series of 10 mL volumetric flasks. To each flask, 1 mL of *p*-dimethylaminobenzaldehyde was added and shaken well at room temperature. The formation of Schiff base was confirmed by measuring its $\lambda_{max}$ at 451 nm ($\lambda_{max}$ of drug was obtained at 272 nm). After 5 min, 0.5 mL of Co(II) solution was added and the solutions were diluted to 10 mL by using ethanol. The absorbance of the green colored complex was measured at 672 nm against reagent blank (Fig. 1). The




**Schiff Base** + **Co(II) Chloride** ⟶ **Green Colored Co(II) Complex**

(R = 4-N(Me)$_2$, 3-OMe-4-OH)

**Scheme 1** Reaction of *p*-dimethylaminobenzaldehyde and vanillin with SFD.

**Table 1** Comparison of $\lambda_{max}$ of Schiff bases and Co(II) complexes synthesized from different aryl aldehydes.

| Aldehydes | R | $\lambda_{max}$ for Schiff base (nm) | $\lambda_{max}$ for Co(II) complex (nm) |
|---|---|---|---|
| 4-Dimetylaminobenzaldehyde | 4-N($Me_2$) | 451 | 672 |
| Vanillin | 3-OMe-4-OH | 398 | 665 |
| Syringaldehyde | 3,5-$(OMe)_2$-4-OH | 325 | 553[a] |
| 4-Chlorobenzaldehyde | 4-Cl | 318 | 552[a] |
| 4-Fluorobenzaldehyde | 4-F | 298 | 549[a] |
| 4-Nitrobenzaldehyde | 4-$NO_2$ | 315 | 554[a] |
| 4-Bromobenzaldehyde | 4-Br | 311 | 552[a] |

[a] Wavelength comparable to the $\lambda_{max}$ of Co(II).

amount of Sulfadoxine present in the sample was computed from the calibration curve.

### 2.4. Determination of sulfadoxine by using vanillin (method B)

Aliquots of sulfadoxine containing 20.00–100 μg $mL^{-1}$ were transferred into a series of 10 mL volumetric flasks. To each flask, 1 mL of vanillin was added and shaken well at room temperature. The formation of Schiff base was confirmed by measuring its $\lambda_{max}$ at 398 nm ($\lambda_{max}$ of drug was obtained at 272 nm). After 5 min, 0.5 mL of Co(II) solution was added and solutions were diluted to 10 mL by using ethanol. The absorbance of the green colored complex was measured at 665 nm against reagent blank (Fig. 1). The amount of Sulfadoxine present in the sample was calculated from the calibration curve.

### 2.5. Preparation of pharmaceutical formulation

Commercial tablets (Reziz forte) of sulfadoxine were analyzed by proposed methods. Tablets' weight equivalent to 750 mg of sulfadoxine was crushed thoroughly in a mortar and dissolved in 20 mL of 2 M hydrochloric acid and diluted to 100 mL by using ethanol. The solution was filtered through a Whatman filter paper No. 41 and diluted quantitatively with ethanol to obtain a suitable concentration for the analysis.

## 3. Results and discussion

Compounds containing an azomethine group (–CH═N–), known as Schiff bases are formed by the condensation of a primary amine with a carbonyl compound (Arulmurugan et al., 2010; Olajire and Offiong, 2009). In the present work, the drug Sulfadoxine which contains the primary amino group, first reacted with aryl aldehydes to form the corresponding Schiff bases (Scheme 1). But, the formed Schiff bases exhibited their absorbance maxima at lower wavelengths (Table 1). However, it is well known that Schiff bases are good ligands for the preparation of complexes (Aliyu and Mohammed, 2009). So, the Schiff bases of sulfadoxine are further treated with $CoCl_2 \cdot 6H_2O$ to form green colored Co(II) complexes.

### 3.1. Selection of aryl aldehydes

Different aryl aldehydes are tested, out of which *p*-dimethylaminobenzaldehyde and vanillin gave stable green colored

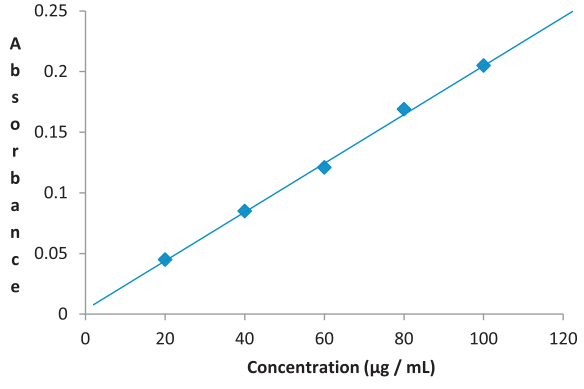



**Figure 2** Calibration curve for method A.

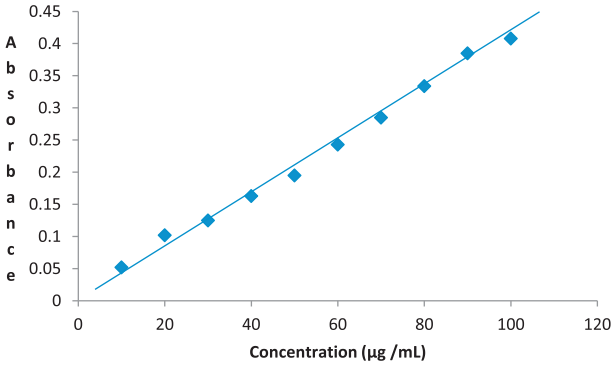



**Figure 3** Calibration curve for method B.

**Table 2** Spectral and Statistical data for the determination of SFD.

| Parameters | Method A | Method B |
|---|---|---|
| $\lambda_{max}$ nm | 672 | 665 |
| Beer's law limits (μg/ml) | 40.00–100.00 | 20.00–100.00 |
| Molar absorptivity (L $mol^{-1}$ $cm^{-1}$) | $0.1264 \times 10^4$ | $0.6982 \times 10^4$ |
| Sandell's sensitivity (μg $mL^{-1}$/0.001 abs units) | 0.2453 | 0.4444 |
| Limit of detection[a] (μg $mL^{-1}$) | 0.2686 | 0.3465 |
| Limit of quantification [a] (μg $mL^{-1}$) | 0.8139 | 0.3465 |
| Slope (*b*) | 0.0043 | 0.0020 |
| Intercept (*a*) | −0.0139 | 0.0038 |
| Correlation coefficient (*r*) | 0.9972 | 0.9990 |

[a] Limit of detection calculated according to ICH guidelines.

**Table 3** Evaluation of accuracy and precision.

| Methods | Amount taken ($\mu g\ mL^{-1}$) | Amount found[a] ($\mu g\ mL^{-1}$) | RE (%) | SD ($\mu g\ mL^{-1}$) | RSD (%) |
|---|---|---|---|---|---|
| Method A | 40.00 | 39.71 | 0.72 | 0.31 | 0.78 |
| | 50.00 | 49.79 | 2.10 | 0.73 | 1.46 |
| | 60.00 | 59.94 | 0.10 | 0.54 | 0.90 |
| Method B | 20.00 | 20.07 | −0.35 | 0.56 | 2.82 |
| | 40.00 | 39.21 | 1.97 | 0.18 | 0.45 |
| | 60.00 | 59.99 | 0.01 | 0.51 | 0.85 |

[a] Mean value of five determinations.

complexes (Table 1). The color stability of these complexes may be due to the presence of electron donating groups in *p*-dimethylaminobenzaldehyde (–$NMe_2$) and vanillin (–OH and –OMe) which helps the formation of Co(II) complexes. Other aryl aldehydes do not form any colored complexes.

### 3.2. Stability of color complexes

The reaction is carried out at room temperature. It has taken around 5 min for the complete color development. After the color development absorbance of the complex is found to be constant up to 6 h.

### 3.3. Beer's law and sensitivity

Under optimum experimental conditions, linear relations are found between absorbance and concentration of Sulfadoxine in the range of 40.00–100.00 ug $mL^{-1}$ (method A, Fig. 2) and 20.00–100.00 ug $mL^{-1}$ (method B, Fig. 3). Correlation coefficient, intercept and slope for the calibration curve are summarized in Table 2. The small values of Sandell's sensitivity parameters indicate high sensitivity of the proposed methods. The limit of detection (LOD) and the limit of quantification (LOQ) are obtained as per the ICH guidelines [16] from the expression

$LOD = 3.3\sigma/S$ and $LOD = 10\sigma/S$

$\sigma$ = Standard deviation of the blank.

$S$ = Slope of the calibration curve.

### 3.4. Accuracy and precision

In order to study the accuracy and precision of the proposed methods, three concentrations of pure Sulfadoxine within the linearity range are analyzed, each determination being repeated five times and the percentage relative standard deviation (% RSD) is found to be less than 2%. Accuracy of the proposed methods is measured by calculating the percentage relative error (% RE) and is found to be less than 3%. The results of this study indicate the high accuracy and precision of the methods. Detailed results are given in Table 3.

**Table 4** Result of assay of formulation by the proposed method.

| Labeled amount (mg) | Found[a] ± SD using method A | Found ± SD using method B |
|---|---|---|
| 750 | 758 ± 0.63<br>$t$ = 0.3052 | 752 ± 0.73<br>$t$ = 0.1409 |

Tabulated $t$ value at 95% confidence level is 2.7.

[a] Mean value of five determinations.

### 3.5. Interference study

The effects of a wide range of excipients which are usually present in the formulations are studied. It is found that the proposed method can be successfully applied for the determination of sulfadoxine in various pharmaceutical formulations without any analytical problem due to the tablet excipients such as glucose, starch and lactose.

## 4. Application to analysis of tablets

The proposed methods are successfully applied to the determination of sulfadoxine in different pharmaceutical solid dosage forms. The results (Table 4) show that Student's $t$ test values at 95% confidence level are less than the theoretical values, which confirmed the good accuracy of the methods. It is also clear that the result obtained from the proposed methods is in good agreement with those obtained from the reported methods.

## 5. Conclusion

The proposed spectrophotometric methods are rapid and easily applicable for the determination of sulfadoxine in tablets. The proposed methods are free from critical experimental conditions and complicated procedures such as heating or extraction steps. The reagents used in the proposed methods are cheap, readily available and the procedures do not involve any tedious sample preparation. These advantages encourage the application of the proposed methods in routine quality control analysis of sulfadoxine in pharmaceutical formulations.

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